

### **Relevant Products**

#D2000G Borealis - V2R (Vasopressin Type 2 Receptor) Assay Kit

#### Materials included

- Borealis Sensor: Green Fluorescent Arrestin Sensor in BacMam (Product #D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment..

- Vasopressin 2 Receptor (V2R) in BacMam (#Z0620N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin II Type I Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H20, Cayman Chemical, #17150

Ang II can be used to stimulate arrrestin recruitment through the positive control, the AT1R receptor.

## **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

### QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

# **Biosafety**

BacMam is the modified baculovirus, *Autographa californica*, AcMNPV. Baculovirus is pseudotyped to infect mammalian cells, but it cannot replicate in the cells and its genome is silent in mammalian cells. While it should be handled carefully, in a sterile environment, it is classified as a BSL-1 reagent. The NIH Guidelines for Research on Recombinant DNA Molecules should be consulted for laboratory safety procedures. Shipment contains sodium butyrate which may cause eye, skin irritation or respiratory irritation.

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MADE IN THE USA

Patent Pending: PCT/US2019/044165

[WO2020028381A1]



### **Relevant Products**

#D2010G Borealis - AT1R (Angiotensin II-Type I Receptor) Assay Kit

## Materials included

- Borealis Sensor: Green Fluorescent Arrestin Sensor in BacMam (Product #D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment..

- Angiotensin II Type I Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin II Type I Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H20, Cayman Chemical, #17150

Ang II can be used to stimulate arrrestin recruitment through the positive control, the AT1R receptor.

# **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

# QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

### **Biosafety**

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Patent Pending: PCT/US2019/044165

[WO2020028381A1]



### **Relevant Products**

#D2020G Borealis - GLP-1R (Glucagon-like Peptide 1 Receptor) Assay Kit

## **Materials included**

- Borealis Sensor: Green Fluorescent Arrestin Sensor in BacMam (Product #D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment..

- GLP-1 receptor (GLP-1R) in BacMam (#Z0600N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- GRK5 in BacMam (#K0005N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

For some GPCRs, addition of a G protein-coupled receptor kinase will further optimize the assay.

Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin II Type I Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

Angiotensin II (Ang II) 5mM, in H20, Cayman Chemical, #17150

Ang II can be used to stimulate arrrestin recruitment through the positive control, the AT1R receptor.

# Storage

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

### QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

## **Biosafety**

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Patent Pending: PCT/US2019/044165

[WO2020028381A1]



### **Relevant Products**

#D2030G Borealis - MOR (μ-Opioid Receptor) Assay Kit

## Materials included

- Borealis Sensor: Green Fluorescent Arrestin Sensor in BacMam (Product #D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment..

- Mu Opioid receptor (OPRM1) in BacMam (#Z0720N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- GRK2 in BacMam (#K0002N) ~ 2x1010 VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin II Type I Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H20, Cayman Chemical, #17150

Ang II can be used to stimulate arrrestin recruitment through the positive control, the AT1R receptor.

# **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

# QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

### **Biosafety**

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Patent Pending: PCT/US2019/044165

[WO2020028381A1]



## **Relevant Products**

#D2040G Borealis - B2AR (β2 Adrenergic Receptor) Assay Kit

## **Materials included**

- Borealis Sensor: Green Fluorescent Arrestin Sensor in BacMam (Product #D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment..

- β2 Adrenergic receptor (B2AR) in BacMam (#Z0500N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- GRK2 in BacMam (#K0002N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin II Type I Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

Angiotensin II (Ang II) 5mM, in H20, Cayman Chemical, #17150

Ang II can be used to stimulate arrrestin recruitment through the positive control, the AT1R receptor.

## **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

#### QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

## **Biosafety**

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Patent Pending: PCT/US2019/044165

[WO2020028381A1]



### **Relevant Products**

#D2050G Borealis - D1R (D1 Dopamine Receptor) Assay Kit

## **Materials included**

- Borealis Sensor: Green Fluorescent Arrestin Sensor in BacMam (Product #D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment..

- D1 Dopamine receptor (D1R) in BacMam (#Z0100N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- GRK2 in BacMam (#K0002N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin II Type I Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H20, Cayman Chemical, #17150

Ang II can be used to stimulate arrrestin recruitment through the positive control, the AT1R receptor.

## Storage

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

### QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

# **Biosafety**

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Patent Pending: PCT/US2019/044165

[WO2020028381A1]



### **Relevant Products**

#D2060G Borealis - OTR (Oxytocin Receptor) Assay Kit

## **Materials included**

 Borealis Sensor: Green Fluorescent Arrestin Sensor in BacMam (Product #D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment..

- Oxytocin receptor (OTR) in BacMam (#Z0900N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin II Type I Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H20, Cayman Chemical, #17150

Ang II can be used to stimulate arrrestin recruitment through the positive control, the AT1R receptor.

# **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

### QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

## **Biosafety**

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Patent Pending: PCT/US2019/044165

[WO2020028381A1]



## **Relevant Products**

#D2070G Borealis - 5-HT2AR (Serotonin 5-HT2A Receptor) Assay Kit

## Materials included

 Borealis Sensor: Green Fluorescent Arrestin Sensor in BacMam (Product #D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment..

- Serotonin 5-HT2A Receptor (5-HT2AR) in BacMam (#Z1100N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- GRK3 in BacMam (#K0003N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin II Type I Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H20, Cayman Chemical, #17150

Ang II can be used to stimulate arrrestin recruitment through the positive control, the AT1R receptor.

## Storage

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

### QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

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Patent Pending: PCT/US2019/044165

[WO2020028381A1]



### **Relevant Products**

#D2080G Borealis - KOR (Kappa Opioid Receptor) assay kit

### Materials included

- Borealis Sensor in BacMam (#D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Green fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment.

- Kappa Opioid Receptor (OPRK1) in BacMam (#Z0710N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- GRK5 in BacMam (#K0005N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin 1 Type II Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H<sub>2</sub>0, Cayman Chemical, #17150

Ang II can be used to stimulate arrestin recruitment through the positive control, the AT1R receptor.

# **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

## QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

# **Biosafety**

BacMam is the modified baculovirus, *Autographa californica*, AcMNPV. Baculovirus is pseudotyped to infect mammalian cells, but it cannot replicate in the cells and its genome is silent in mammalian cells. While it should be handled carefully, in a sterile environment, it is classified as a BSL-1 reagent. The NIH Guidelines for Research on Recombinant DNA Molecules should be consulted for laboratory safety procedures. Shipment contains sodium butyrate which may cause eye, skin irritation or respiratory irritation.

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MADE IN THE USA

Patent Pending: PCT/US2019/044165

[WO2020028381A1]



### **Relevant Products**

#D2090G Borealis - PAR2 (Proteinase-activated receptor) assay kit

#### Materials included

- Borealis Sensor in BacMam (#D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Green fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment.

- Proteinase-activated Receptor (PAR2) in BacMam (#Z1200N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin 1 Type II Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H<sub>2</sub>0, Cayman Chemical, #17150

Ang II can be used to stimulate arrestin recruitment through the positive control, the AT1R receptor.

# **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

## QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

# **Biosafety**

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MADE IN THE USA

Patent Pending: PCT/US2019/044165

[WO2020028381A1]



### **Relevant Products**

#D2100G Borealis - DOR (Delta Opioid Receptor) assay kit

#### Materials included

- Borealis Sensor in BacMam (#D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Green fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment.

- Delta Opioid Receptor (OPRD1) in BacMam (#Z0740N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- GRK5 in BacMam (#K0005N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin 1 Type II Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H<sub>2</sub>0, Cayman Chemical, #17150

Ang II can be used to stimulate arrestin recruitment through the positive control, the AT1R receptor.

# **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

## QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

# **Biosafety**

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Patent Pending: PCT/US2019/044165

[WO2020028381A1]



### **Relevant Products**

#D2110G Borealis - LPA1R (Lysophosphatidic Acid Receptor 1) assay kit

#### Materials included

- Borealis Sensor in BacMam (#D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Green fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment.

- Lysophosphatidic Acid Receptor 1 (LPA1R) in BacMam (#Z1300N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin 1 Type II Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H<sub>2</sub>0, Cayman Chemical, #17150

Ang II can be used to stimulate arrestin recruitment through the positive control, the AT1R receptor.

# **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

## QA/QC

BacMam stocks are tested for sterility. Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

# **Biosafety**

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Patent Pending: PCT/US2019/044165

[WO2020028381A1]



### **Relevant Products**

#D2120G Borealis - PTH1R (Parathyroid Hormone 1 Receptor) assay kit

#### Materials included

- Borealis Sensor in BacMam (#D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Green fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment.

- Parathyroid Hormone 1 Receptor (PTH1R) in BacMam (#Z1400N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin 1 Type II Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H<sub>2</sub>0, Cayman Chemical, #17150

Ang II can be used to stimulate arrestin recruitment through the positive control, the AT1R receptor.

# **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

## QA/QC

BacMam stocks are tested for sterility. Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

# **Biosafety**

BacMam is the modified baculovirus, *Autographa californica*, AcMNPV. Baculovirus is pseudotyped to infect mammalian cells, but it cannot replicate in the cells and its genome is silent in mammalian cells. While it should be handled carefully, in a sterile environment, it is classified as a BSL-1 reagent. The NIH Guidelines for Research on Recombinant DNA Molecules should be consulted for laboratory safety procedures. Shipment contains sodium butyrate which may cause eye, skin irritation or respiratory irritation.

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Patent Pending: PCT/US2019/044165

[WO2020028381A1]



## **Relevant Products**

#D2130G Borealis - 5-HT2CR (Serotonin 5-HT2C Receptor) Assay Kit

## **Materials included**

- Borealis Sensor: Green Fluorescent Arrestin Sensor in BacMam (Product #D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment..

- Serotonin 5-HT<sub>2C</sub> Receptor (5-HT2CR) in BacMam (#Z1800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin II Type I Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H20, Cayman Chemical, #17150

Ang II can be used to stimulate arrrestin recruitment through the positive control, the AT1R receptor.

# **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

### QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

## **Biosafety**

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MADE IN THE USA

Patent Pending: PCT/US2019/044165

[WO2020028381A1]

Revision date: 3/2/2023



### **Relevant Products**

#D2140G Borealis - Glucagon Receptor (GCGR) Assay Kit

## Materials included

 Borealis Sensor: Green Fluorescent Arrestin Sensor in BacMam (Product #D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment..

- Glucagon Receptor (GCGR) in BacMam (#Z2000N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product # B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin II Type I Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H20, Cayman Chemical, #17150

Ang II can be used to stimulate arrrestin recruitment through the positive control, the AT1R receptor.

# **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

### QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. After 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

## **Biosafety**

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MADE IN THE USA

Patent Pending: PCT/US2019/044165

[WO2020028381A1]

Revision date: 5/22/2023



### **Relevant Products**

#D2150G Borealis - MC4R (Melanocortin 4 Receptor) assay kit

#### Materials included

Borealis Sensor in BacMam (#D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Green fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment.

- Melanocortin Receptor 4 (MC4R) in BacMam (#Z2239N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product #B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin 1 Type II Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H<sub>2</sub>0, Cayman Chemical, #17150

Ang II can be used to stimulate arrestin recruitment through the positive control, the AT1R receptor.

# **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

### QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. Iff applicable, after 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

### **Biosafety**

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MADE IN THE USA

Patent Pending: PCT/US2019/044165

[WO2020028381A1]



### **Relevant Products**

#D2160G Borealis - CCR5 (C-C Chemokine Receptor Type 5) assay kit

#### Materials included

- Borealis Sensor in BacMam (#D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Green fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment.

- C-C Chemokine Receptor Type 5 (CCR5) in BacMam (#Z1010N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- GRK5 in BacMam (#K0005N) ~ 2x1010 VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product #B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin 1 Type II Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H<sub>2</sub>0, Cayman Chemical, #17150

Ang II can be used to stimulate arrestin recruitment through the positive control, the AT1R receptor.

## **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

### QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. Iff applicable, after 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

## **Biosafety**

BacMam is the modified baculovirus, *Autographa californica*, AcMNPV. Baculovirus is pseudotyped to infect mammalian cells, but it cannot replicate in the cells and its genome is silent in mammalian cells. While it should be handled carefully, in a sterile environment, it is classified as a BSL-1 reagent. The NIH Guidelines for Research on Recombinant DNA Molecules should be consulted for laboratory safety procedures. Shipment contains sodium butyrate which may cause eye, skin irritation or respiratory irritation.

For Research Use Only. Not recommended for use or sale in human or animal diagnostic or therapeutic products. This product contains no substances which at their given concentration are considered to be hazardous to health, however we recommend handling with care. Wear impervious gloves and eye protection when handling. Do not ingest.

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### **Relevant Products**

#D2170G Borealis - PAR1 (Protease-activated Receptor 1) assay kit

#### Materials included

- Borealis Sensor in BacMam (#D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Green fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment.

- Protease-activated Receptor 1 in BacMam (#Z1219N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product #B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin 1 Type II Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H<sub>2</sub>0, Cayman Chemical, #17150

Ang II can be used to stimulate arrestin recruitment through the positive control, the AT1R receptor.

# **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

## QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. Iff applicable, after 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

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### **Relevant Products**

#D2180G Borealis - Apelin Receptor assay kit

#### Materials included

Borealis Sensor in BacMam (#D2222G) ~ 3x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).

Green fluorescent sensor that changes in fluorescence intensity in response to arrestin recruitment.

- Apelin Receptor in BacMam (#Z2309N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01).
- Sodium Butyrate (Sigma Aldrich product #B5887) 500 mM in H<sub>2</sub>O.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types.

 Angiotensin 1 Type II Receptor (AT1R) in BacMam (#Z0800N) ~ 2x10<sup>10</sup> VG/mL in ESF 921 Insect Cell Culture Medium (Expression Systems #96-001-01)

The AT1R is provided as a positive control for the purpose of assay optimization.

- Angiotensin II (Ang II) 5mM, in H<sub>2</sub>0, Cayman Chemical, #17150

Ang II can be used to stimulate arrestin recruitment through the positive control, the AT1R receptor.

# **Storage**

BacMam vectors should be stored at 4°C and protected from light in the original package. Avoid repeated freeze/thaw cycles. Store HDAC inhibitor at 4°C. Store control agonist at -20°C.

Add sodium butyrate to cultured cells to maintain BacMam expression as needed. Other HDAC inhibitors may work as well or even better in certain cell types. For expression in CHO cells, we recommend valproic acid instead of sodium butyrate. Many cell types maintain expression without an HDAC inhibitor.

## QA/QC

BacMam stocks are tested for sterility Samples are added to rich cell culture media without antibiotic and incubated at 37°C, 5% CO<sub>2</sub>, and checked for bacterial or fungal growth after 5 days. Viral genes (VG) per milliliter (mL) are measured by qPCR with primers specific to VSVG. Check tube label for exact titer value. Viral genomic DNA at multiple dilutions are run in qPCR against a standard curve to generate an average titer for each BacMam stock. Each tube of stock is labeled with VG/mL and a stock keeping unit (SKU) identifier. To test efficacy, serial dilutions are added to cultured HEK 293 cells. Iff applicable, after 24 hours, fluorescent cells are counted to establish transducing units per mL of stock.

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